

CONTROL OF NERVE AGENT-INDUCED SEIZURES IS CRITICAL FOR NEUROPROTECTION AND SURVIVAL

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ABSTRACT

All six nerve agents studied were capable of producing prolonged seizures (*status epilepticus*) and neuropathology, and all 5 tested drugs were capable of terminating seizure activity. This presentation focused on the aspect of the influence of seizure activity, whether it was or was not terminated by any of the drug treatment, on neuropathological consequence and on mortality. Regardless of doses and drug treatment, control of seizure was strongly associated with protection against acute lethality and brain pathology, while failure to stop seizures was associated with increased lethality and more frequent and severe brain pathology. Thus, effective anticonvulsant treatment of a nerve agent casualty is critical to immediate and long-term recovery.

INTRODUCTION

The chemical warfare nerve agents, such as tabun, sarin, soman and VX, are organophosphorus (OP) cholinesterase inhibitors. Exposure to these agents causes a progression of toxic signs (Taylor 1985). In addition to hypersecretions, fasciculations, tremor, convulsions and respiratory distress, prolonged seizures (*status epilepticus*) can begin rapidly after nerve agent exposure in animals and humans. Animal studies show these seizures can result in neuropathology and long-term behavioral deficits if not promptly controlled (McDonough et al. 1995; McDonough and Shih 1997). A combined treatment regimen of prophylaxis and therapy is now generally agreed upon as the most effective medical countermeasure for dealing with the threat of nerve agent poisoning (Dunn and Sidell 1989; Moore et al. 1995; Sidell 1997). Pretreatment with carbamate cholinesterase inhibitors, such as pyridostigmine, binds a small fraction of cholinesterase in the periphery and reversibly shields it from irreversible inhibition by the nerve agent. In the event of poisoning, an anticholinergic drug, such as atropine sulfate, is used to antagonize the effects of excess acetylcholine at muscarinic receptor sites, and an oxime, such as pralidoxime chloride (2-PAM Cl), is used to reactivate any unaged inhibited enzyme (Moore et al. 1995). However, this treatment regimen does not control the development of nerve agent-induced seizures (McDonough et al. 1999).

Although diazepam or a water soluble prodrug form of diazepam, avizafone (Clement and Broxup 1993), is the drug that has been adopted by most military forces for the immediate treatment of nerve agent seizures (Moore et al. 1995), research has demonstrated that diazepam is not always completely effective in protecting animals against nerve agent-induced neuropathology (Hayward et al. 1990; McDonough et al. 1995). Thus, there has been continuing debate as to whether diazepam is the best drug to use for the treatment of nerve agent seizures (McDonough and Shih 1997). Much of this debate has centered around pharmacokinetic issues, since diazepam, or for that matter any emergency treatment drug used by the military for nerve agent poisoning, must be given intramuscularly (im) by automatic injectors. This is necessitated by the fact that all personnel must be able to receive immediate treatment in the event of exposure. Therefore, there is a continuing need to find better drugs to treat seizures elicited by OP nerve agents. Our research effort was initially focused on soman exposure and protection (Shih et al. 1991; McDonough et al. 1999). However,

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during the past several years this effort has been extended to include many other threat nerve agents, such as tabun, sarin, GF, VR and VX (Shih and McDonough 1999).

We have recently completed a study on the effectiveness of several anticholinergic (atropine sulfate, biperiden HCl, or trihexyphenidyl HCl) and benzodiazepine (diazepam or midazolam) drugs to terminate epileptiform seizures produced by six threat OP nerve agents (tabun, sarin, soman, GF, VX and VR) in a guinea pig model that closely approximates the use of pretreatment and therapy drugs as medical countermeasures for nerve agent exposure (Shih and McDonough 2000). Specifically, anticonvulsant ED₅₀s were determined for each drug treatment at 5 min after the onset of EEG seizures after nerve agent exposure. The animals in these tests thus could be subdivided into those in which seizure activity was successfully terminated by the drug treatment and those in which seizure activity continued. The present report describes the analysis of the relationship between seizure termination and neuropathology, and between seizure termination and lethality.

Male Hartley guinea pigs (CrI: (HA) BR COBS; Charles River Labs, Kingston, NY, USA) of 250-300 g body weight at the start of the study served as subjects. They were prepared approximately one week before experimentation with cortical stainless steel screw electrodes using previously described procedures (McDonough et al. 1999). EEG recordings were made using QND software and amplifiers supplied by Neurodata Inc. (Pasadena, CA) (low frequency filter = 0.3 Hz; high frequency filter = 40 Hz; sampling rate = 128 Hz) and displayed on a computer monitor. During EEG recordings, all animals were housed in individual plastic recording chambers that allowed free movement with the exception of the recording leads attached to the electrode connector on the top of the head.

On the day of the experiment, guinea pigs were continuously monitored for EEG activity. After a 15-min recording of baseline EEG measures, animals received pyridostigmine (0.026 mg/kg, im) to produce ~30% whole blood ChE inhibition (Lennox et al. 1985). Thirty min later, animals were challenged with 2 x LD₅₀ subcutaneous (sc) dose of tabun (240 ug/kg), sarin (84 ug/kg), soman (56 ug/kg), GF (114 ug/kg), VR (22 ug/kg) or VX (16 ug/kg). One min after nerve agent challenge, all animals were treated with atropine sulfate (2 mg/kg, im) plus 2-PAM (25 mg/kg, im). Five min after the onset of EEG seizure activity, different doses of the anticholinergic drug atropine sulfate, biperiden HCl, trihexyphenidyl HCl, or the benzodiazepine drug diazepam or midazolam were given intramuscularly. Animals were observed continuously for the first hour following exposure and treatment and periodically thereafter for at least 6 hr. EEGs were monitored continuously throughout this time and for 30 min at 24 hr. Seizure onset was operationally defined as the appearance of ≥10 sec of rhythmic high amplitude spikes or sharp wave activity in the EEG. Each animal was rated as having the seizure terminated (OFF) or not terminated (NOT OFF) based on the overall appearance of the EEG record at the end of the experimental day and during the 24-hr observation. (Note: An animal was rated as OFF if the seizure was terminated and the EEG remained normal at all subsequent observation times.)

Surviving animals at 24 hr after nerve agent exposure were deeply anesthetized with pentobarbital (75 mg/kg, ip) and perfused through the aorta with saline followed by 10% neutral buffered formalin. The brain was blocked, embedded in paraffin, cut 6-10 microns thick, stained with hematoxylin and eosin and then evaluated by a board certified pathologist who was unaware of the experimental history of a given subject. The procedures and criteria used for pathological evaluation have been published (McDonough et al. 1995, 2000). Briefly, six brain areas (cerebral cortex, pyriform cortex, amygdala, hippocampus, caudate nucleus, thalamus) were evaluated in each animal; each area was given a score that described brain lesion severity based on the approximate percentage of tissue involvement: 0=none, 1=minimal, 1-10%; 2=mild, 11-25%; 3=moderate, 26-45%; 4=severe, >45%. For each animal, a total neuropathology score was obtained by summing the scores of the six brain areas. The criterion used to characterize the lesion/pathology was neuronal necrosis, e.g., shrunken eosinophilic neurons with dark, round, pyknotic nuclei.

The proportion of animals surviving as a function of successful control of the seizure, as well as the incidence of neuropathology as a function of seizure control, was evaluated using the Chi-square procedure with Yates correction (Winer, 1971). The latency to seizure termination was evaluated between drugs using the Kruskal-Wallis one-way analysis of ranks. The total neuropathology scores were categorized by whether seizure was turned OFF vs. NOT OFF and by drug and then was evaluated by a two-way analysis of variance.

All six OP nerve agents were capable of inducing brain seizure activity in this model. When drug treatment failed to stop the seizure, epileptiform activity was evident continuously throughout the 6-hr experimental period and could still be observed in some animals 24 hr after nerve agent exposure.

TABLE 1. Acute (24-h) Survival as a Function of Seizure Control.

	24-h Survival		TOTAL
	ALIVE	DEAD	
SEIZURE OFF	411 (96%)	16 (4%)	427
SEIZURE NOT OFF	232 (58%)	169 (42%)	401

Table 1 shows the strong relationship between the control of seizures and protection against the lethal effect of nerve agent exposure. Animals were categorized by seizure outcome (OFF, NOT OFF) and lethality (ALIVE, DEAD) at 24 hrs following nerve agent exposure. Less than 4% of the animals that had their seizures successfully controlled (OFF) died, whereas 42% of animals that failed to have the seizures controlled (NOT OFF) died. This result was highly significant ($\chi^2 = 173.5$, $df = 1$, $p < 0.001$). Analysis of the individual drugs showed this was a consistent and robust finding.

The brains of 593 animals were available for pathological evaluation. All the nerve agents were capable of producing neuropathology under the conditions of this study. No drug completely protected against neuropathology development, but control of seizure clearly had an influence on the incidence and severity of neuropathology (Tables 2 and 3). Animals that had their seizure successfully controlled (OFF) had significantly higher numbers of brains that displayed no neuropathology ($\chi^2 = 81.14$, $df = 1$, $p < 0.001$). Again, analysis of the individual drugs showed that this was a consistent and robust finding with each compound.

TABLE 2. Incidence of Neuropathology as a Function of Seizure Control.

	No pathology	Pathology	TOTAL
SEIZURE OFF	284 (78%)	77 (22%)	361
SEIZURE NOT OFF	55 (24%)	177 (76%)	232

Table 3 displays the average brain lesion scores for individual brain areas. The data are collapsed across all nerve agents and then categorized by whether the seizure was rated OFF or NOT OFF and listed by treatment drug. The data clearly show that failure to control the seizure resulted in significantly greater numbers of animals with brain lesions, and in general the severity of the lesion was greater than in animals in which the seizures were successfully controlled by the treatment drug. The data also show that the cortex and amygdala were the brain areas most likely to experience the greatest damage.

CONCLUSION

The present study shows that all six nerve agents tested can induce prolonged brain seizures (*status epilepticus*) and have the potential for producing neuropathology in our guinea pig model. These results provide strong evidence that the prolonged seizure activity elicited by the nerve agents was the major factor in lesion production (McDonough et al. 1995). Animals that had seizures controlled by the different anticonvulsant treatments (OFF) were significantly more likely to be totally free of neuropathology or, if it did occur, to have it greatly reduced in both incidence and severity. This is in agreement with previous findings in both rodents (Lallement et al. 1994; McDonough et al. 2000) and nonhuman primates (Hayward et al. 1990; Lallement et al. 1997, 1998) that any treatment that can reduce or terminate seizure activity in nerve agent-exposed animals has a protective effect on the development of neuropathology.

TABLE 3. Mean brain lesion score by brain area collapsed across all nerve agents and categorized by seizure control and treatment drug.

Mean Brain Lesion Scores*							
	Drug	Brain Area					
		Cortex	Amygdala	Piriform	Hippocampus	Caudate	Thalamus
OFF	ATR	1.5(6)	1.4(3)	1.8(4)	1.1(8)	1.0(5)	1.7(3)
	BIP	1.1(26)	1.7(10)	1.1(8)	1.1(7)	1.2(5)	1.1(15)
	THX	1.0(5)	3.0(3)	0(0)	1.4(5)	1.0(1)	1.0(1)
	DIZ	1.3(9)	2.0(8)	1.0(5)	0(0)	0(0)	1.0(2)
	MDZ	1.1(7)	2.4(7)	0(0)	1.4(5)	0(0)	1.0(1)
	Total	1.2(53)	2.1(31)	1.2(17)	1.5(25)	1.1(11)	1.1(22)
NOT	ATR	1.2(14)	2.6(11)	1.8(12)	1.0(8)	2.0(8)	1.9(14)
OFF	BIP	3.5(43)	3.8(41)	2.0(38)	1.1(33)	3.0(41)	3.3(34)
	THX	2.9(20)	3.8(21)	1.7(13)	1.3(18)	3.2(18)	2.0(19)
	DIZ	2.8(54)	3.3(55)	1.7(48)	1.0(2)	1.7(19)	1.7(44)
	MDZ	2.3(36)	3.7(33)	1.6(25)	1.5(15)	2.9(21)	1.7(28)
	Total	2.7(167)	3.5(161)	1.8(136)	1.2(76)	2.7(107)	2.1(139)

*number in parenthesis indicate number of animals involved.

Another interesting aspect of these results was the strong association between anticonvulsant effect of the treatment and protection from the acute lethal effects of the nerve agents. Such association has been seen in previous studies from our laboratory with both anticholinergic as well as benzodiazepine drugs using soman challenge in this guinea pig model (McDonough et al. 1999, 2000) and has also been observed in nonhuman primate studies of anticonvulsant treatment of soman exposure (Lallement et al. 1997, 1998). Such an association between mortality and status epilepticus is well recognized in the clinical medical literature (Towne et al. 1994; Krumholz et al. 1995). The fact that control of nerve agent seizures is so strongly linked to protection from the lethal effects of nerve agents may explain the requirement for such high doses of atropine that have been routinely used in studies of the protective effects of carbamate pretreatment (Dirnhuber et al. 1979; Maxwell et al. 1988) or oxime therapies (Melchers et al. 1994; Worek et al. 1994; Koplovitz et al. 1995). These findings lend perspective to the older reports that inclusion of a benzodiazepine to standard atropine and oxime therapy would increase the protective ratios against OP nerve agent exposure (Johnson and Wilcox 1975; Boskovic 1981).

In summary, animals that had seizures successfully terminated by an anticonvulsant drug were significantly more likely to survive and were more likely to be totally protected from or experience only mild forms of brain pathology. These data indicate the importance of immediate anticonvulsant drug treatment in combination with traditional atropine and oxime therapy in OP nerve agent poisoning.

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